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REGISTRATION LETTER

Ref: JU/ PHD_Registration/2013/13/903 **Date:** 29th May 2013

Mr. Ajay Pal
Research Scholar
JECRC University, Jaipur

Dear Mr. Ajay Pal,

The University Research Committee (URC) has approved your research proposal, entitled- **"ROLE OF VESICULAR ARBUSCULAR MYCORRHIZAL BIODIVERSITY IN RESTORATION OF SOIL FERTILITY OF ARID LAND IN JAIPUR REGION "** on 6th April 2013 for registration for Ph.D. at JECRC University.

The details as approved by URC are as follows:

1.	Name of the Guide:	Dr. Sonali Pandey
2.	Title of Research Work:	ROLE OF VESICULAR ARBUSCULAR MYCORRHIZAL BIODIVERSITY IN RESTORATION OF SOIL FERTILITY OF ARID LAND IN JAIPUR REGION
3.	University Registration No.:	**1203004003**
4.	Date of Registration:	06-04-2013
5.	Type of Registration:	Full Time Scholar
6.	Duration of Research Work:	Minimum 2 Years, Maximum 5 Years
7.	Entrance Examination:	Cleared in July 2012

You have to undergo the following coursework within One year of the Registration:

i. Research Methodology: 4 credits
ii. Two Seminars (of 2 Credits each) as follows-
   1. Role of Glomalin in improving Soil Fertility
   2. In Vitro Culture of VAM

You'll abide by the rules and regulations of JECRC University during the period of your research work.

Yours,

Dr. D.P. Misra
Registrar, JECRC University.

C.C.:
1. Ajay Pal
2. Dr. Sonali Pandey, Guide of the Student
3. PhD Course Coordinator, JU
4. Personal File of the Student

ROLE OF VESICULAR ARBUSCULAR MYCORRHIZAL BIO-DIVERSITY IN RESTORATION

OF SOIL FERTILITY OF ARID LAND IN JAIPUR REGION

Research Protocol

For Registration for Ph.D. Programme

By

Ajay pal

Under the Guidance of:

Dr. Sonali Pandey

(Associate Professor, Department of Microbiology)

JECRC University, Jaipur

2013

JECRC UNIVERSITY, JAIPUR

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INTRODUCTION

Agriculture is a critical sector of all Indian economy. The increased use of chemical fertilizers in agriculture no doubt increased the food grain production and helped the country in achieving self-sufficiency in food grains. However, it has also produced many harmful effects. The experience from BRIC countries indicate that 1% increase in growth in agriculture is at least 2-3 times more effective in reducing poverty, than the same growth emanating from non agriculture sectors. It may be noted that in last two-five years plans it is clearly mentioned that for the economy to grow at 9% it is important that agriculture should grow at least 4% per annum. With the increase in population the compulsion will be not only to stabilize agricultural production, but also to increase it further in a sustainable manner. Indian arid zone occupies an area of about 0.3 million square Km. Ninety percent of which is confined to North East India covering most of Western Rajasthan, part of Gujarat and small portion of Punjab and Haryana state.

The total land area of Rajasthan is 3,42,239 sq. km out of which 45.25% is characterized as wasteland. Large portion of this land were productive but due to man-made deforestation, cattle pressure and improper water management they have turned out to be wasteland. With an increase pressure of population and a decreasing per capita availability of cultivable land there is a need to enhance cropping intensity without compromising the land productivity (Kotia and Kumar, 2001a).Along with this, today's global issue is sustainable development by maintaining healthy environment. The degradation of soil by chemical fertilizers, fungicides, pesticides and weedicide has created a chemophobia to scientific community. An alternative to chemical fertilizers are biofertilizers to enhance the crop productivity. Biofertilizers aim at cultivating the soil and raising the crop in such a way so as to keep the soil alive and healthy.

The agricultural sustainability could be viewed as maximum plant production with minimum soil loss. The establishment of plant cover is the most important step in restoration of degraded areas. The soils of disturbed sites are frequently low in available nutrients which result in poor growth or no growth of plants. A recent discussion on conventional versus organic production systems highlighted how the green revolution helped to meet the needs of an ever increasing population but at the prize of environmental pollution.

The conventional agriculture has traditionally focused on the potential of mycorrhizae fungi to improve crop yield. Vesicular-arbuscular mycorrhizal (VAM) fungi form endomycorrhizal associations with the root of 70-90% of all known vascular plant species. Vesicular arbuscular mycorrhizal fungi benefit their host plant by improving the uptake of water, minerals and particularly the poorly mobile ion phosphorus in the soil. The fungus has shown to improve the tolerance of plant to drought stress (Barea and Jeffries, 1995). It is established fact that the efficiency of phosphatic fertilizers is very low (15-20%) due to its fixation in acidic and alkaline soils, and unfortunately both soil types are predominant in India accounting more than 34% acidity affected and more than 7 million hectares of productive land are affected with salinity (Yawalkar *et al.,* 2000).

The tremendous advances in research on mycorrhizal physiology and ecology over the past years have led to a greater understanding of the multiple roles of Arbuscular mycorrhizal Fungi in the ecosystem.

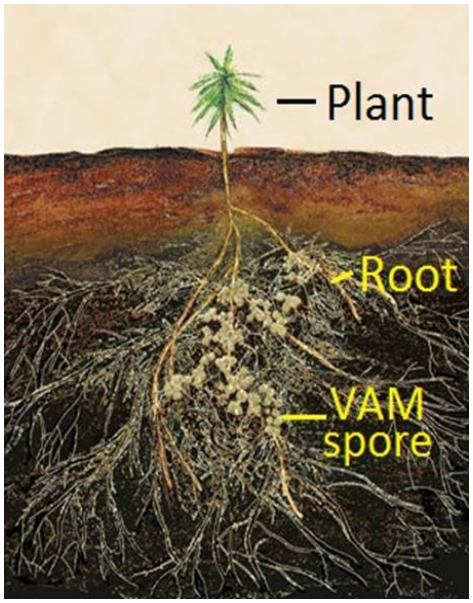


Fig.(i)- VAM fungus with plant

Fig.(ii)- VAM fungus on root

Arbuscular mycorrhizal fungi are important soil organism belonging to the phylum Glomeromycota and form symbiotic association with the majority of terrestrial plant species (Mosse, 1973). In this symbiosis, the host plant provides the fungus with soluble carbon sources which enhance the uptake of certain nutrients by root and increases plants resistance against pathogens, improves plant tolerance to environmental stresses such as drought and accelerates plant establishment. Arbuscular mycorrhizal fungi play a critical role in vegetation succession of ecosystem, plant diversification and productivity, restoration and reestablishment of degraded ecosystems (Dhillion and Gardsjord, 2004)

The mycorrhizae are the feeder root of plant growing in nature and are beneficial to their host plant. In host plant these infected feeder roots are transformed into unique morphological structures called mycorrhizae that is called ‘fungus roots’ (Greek word). A recent analysis of phylogenetic distribution of mycorrhizae occurrence among different species of land plants shows that the arbuscular mycorrhiza are the predominant and ancestral type of mycorrhizae.

The soils of disturbed site are frequently low in available nutrient which results in poor growth of plants. Arbuscular mycorrhizal fungi are known to enhance fertility of soil and inoculation of plants with them in waste land improves the absorption of nutrient from soil and significantly increases the growth of plants.

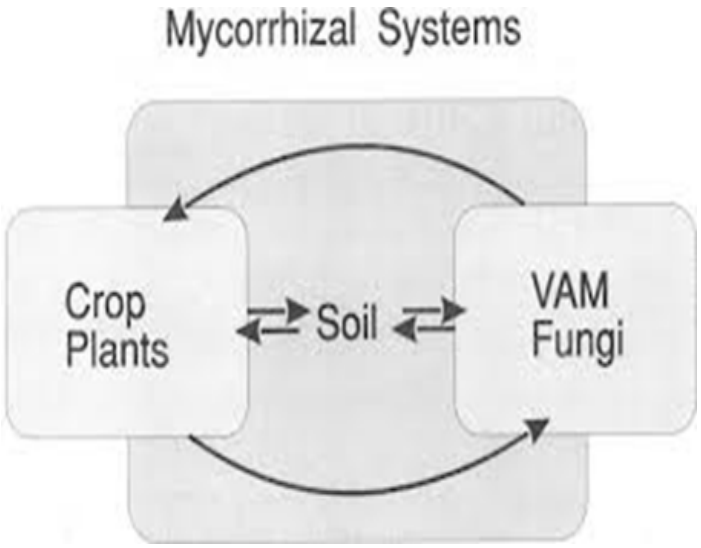


Fig.(iii)-Symbiotic association with VAM fungi and plant

The rehabilitation and reclamation of waste land by vesicular arbuscular mycorrhiza and the establishment of a plant cover is the important step in restoration of degraded areas (Charles *et al.,* 2006*).* Transplanting native plants inoculated with vesicular arbuscular mycorrhiza (VAM) is a good system to establish shrubs and trees in eroded semi-arid land (Caravaca *et al.,* 2003).

The Arbuscular mycorrhizal fungal hyphae are related with the production of a glycoprotein called 'Glomalin' which has a cementing capacity to maintain soil particles together and is mainly involved in soil aggregation. The discovery of glomalin was reported by Upadhyaya *et al* (1996), which are accumulated in soil (Rillig *et al.,* 2001). Glomalin (glue like properties) or glomalin related soil protein (GRSP) is a glycoprotein which sloughs off from the fungal hyphae and accumulate in soil. The hyphae of vesicular arbuscular mycorrhizal fungi are considered to be primary soil aggregators and there is a positive correlation between Arbuscular mycorrhizal fungal hyphae and soil stability in natural ecosystem (Borie *et al.,* 2008). The benefits of glomalin is, in increasing aggregate stability which leads to better soil structure and in turn leads to increase water holding capacity of soil and better plant production.

Against this background of information and utility of VAM in reestablishment of soil fertility, the present investigation will be conducted in Jaipur region.

REVIEW OF LITERATURE

The advances in research on mycorrhizal physiology and ecology over past few years have led to a greater understanding of the multiple roles of mycorrhizal fungi in ecosystem.

Mosse (1973) first reported the beneficial effect of vesicular arbuscular mycorrhizae (VAM) fungi, on plant growth. Vesicular arbuscular mycorrhizal fungi are important soil organism which belongs to phylum Glomeromycota and form symbiotic associations with majority of terrestrial plant species (Smith and Read, 2008).

Vesicular arbuscular mycorrhizal beneficial symbiotic association with root increases the plant ability to absorb phosphorus (P), minor elements and water uptake (Hayman, 1982, Harley and Smith, 1983).Mycorrhizal fungi are benefited with carbon substrate from plants and in turn the plants are provided with nutrients especially phosphorous compounds from soil solution through the hyphal network of the fungi apart from increased absorptive surface area of the roots (Ravarkar *et al.,* 2000).

Gaur and Rana (1990) reported the inoculation of wheat with two vesicular arbuscular mycorrhizal fungi (*Glomus fasciculatum* and *Gigaspora margarita*) which gave improved dry matter yield. There is an increase in vesicular arbuscular mycorrhizal fungus sporulation under salt stress conditions (Bhaskaran *et al.,* 1997).

Vesicular arbuscular mycorrhizal colonization is suppressed by the presence of fluorescent *Pseudomonas* spp. resulting in a reduced photosynthate loss from the plant and a corresponding increase in plant biomass and increased nutrient level. Maximum yield was obtained with microbial inoculation and fertilizers together (Dhillion, 1992).Miller and Jastrow(1992) had reported that mycorrhizal hyphae are involved as an important cementing agent in forming soil aggregate and its stability.

Wright and Upadhyaya (1996) reported Glomalin, a glycoprotein which is produced by arbuscular mycorrhizal fungi and glomalin in soil is quantified as glomalin-related soil protein (GRSP).

Heijden *et al.* (1998) reported that, the community of vesicular arbuscular mycorrhizal fungi determines the plant community structure of an area due to the response of individual plant species by single or multiple species of arbuscular mycorrhizal fungi.

The absence of mycorrhizal fungi on degraded landscapes can also slow plant growth in early succession (Jeffries *et al*., 2003).

Takacs and Voros (2003) reported the role of arbuscular mycorrhizal fungi in the water uptake and nutrient supplies to the host plant. Vesicular arbuscular mycorrhizal fungi improve plant mineral nutrition and are therefore useful for agricultural production. It has been reported that plant roots alone may be incapable of taking up phosphate ions that are demineralized in soil with a basic pH. The mycelium of the mycorrhizal fungus can, however, access these phosphorus sources and make them available to the plants they colonize (Liu*et al.,* 2006).

A number of interacting factors affect the successful colonization of VAM fungi are pH, soil nutrients, organic matter, moisture, temperature, and the age of disturbed sites (Mukhopadhyay and Maiti, 2009).

The Arbuscular mycorrhizal fungi can alleviate the unfavorable effects on plant growth due to stresses such as heavy metals, soil compaction, salinity and drought (Miransari, 2010).

Allen (2011) investigated the fungal interface between the soil and plant system and found that the mycorrhizal fungi are able to utilize hydraulic-lifted water from plants and obtain nutrients from soil in extremely dry surface soils.

Porcel *et al.*, (2012) investigated the salinity stress alleviation effect on plants using arbuscular mycorrhizal fungi. The arbuscular mycorrhizal symbiosis under salinity is a promising field and helps us to study new mechanisms involved in the enhanced tolerance of mycorrhiza infected plants to salt stress.

STATEMENT OF THE PROBLEM

Role of vesicular arbuscular mycorrhizal biodiversity in restoration of soil fertility of arid land in Jaipur region.

Definition:-

Vesicular Arbuscular Mycorrhizal (VAM) fungi- A group of soil fungi that grows on vascular plant roots. (mykos=fungus; rhizal= root)

Arbuscular refers to arbuscule- a structure found in cortical cells of roots.

OBJECTIVESOF THE STUDY

1. To survey and collection of soil samples from the rhizosphere of plants grown in farmlands of Jaipur region.

2. To isolate and identify different vesicular arbuscular mycorrhizal fungal species.

3. To inoculate selected vesicular arbuscular mycorrhizal species on selected winter and summer crops.

4. To study morphological, biochemical and histochemical parameters on plants colonized with vesicular arbuscular mycorrhizal species.

5. To select and recommend indigenous vesicular arbuscular mycorrhizal species suitable to the agro-climatic condition of Jaipur.

SCOPE OF THE STUDY

At present Scenario use of biofertilizer is still considered as a supplement not a substitute for maximizing the agro-system stability. After studying the morphological, biochemical and histochemical parameters in vesicular arbuscular mycorrhiza infected plants, in this study we will investigate the importance of VAM in restoration and reestablishment of degraded ecosystems.

LIMITATIONS

The limitations of work are-

(I) The vesicular arbuscular mycorrhizal association with plant is related to physiochemical and biological properties of barren land. And our work is confined only to the Jaipur region.

(II) The experimentation will be restricted only on selected Vesicular arbuscular mycorrhizae fungal species.

SIGNIFICANCE OF THE STUDY

The indiscriminate use of synthetic fertilizers causes environmental pollution and contamination of the soil making the crop more prone to disease and reduces the soil fertility. The long term use of VAM biofertilizer is economical, ecofriendly, more efficient and productive in providing natural fertility to the soil.

METHODOLOGY

Following procedure will be followed for the experimentation-

- **Survey and collection of soil samples:** The two sampling sites (Farmlands at Tonk road and Delhi Road) are selected. Each farmland will be divided into four different zones. Soil samples will be collected randomly from each zone at a depth of 0-15 cm after scrapping the top 1cm layer of soil. The samples collected from different places in each sites are pooled and homogenized. The collected soil and root samples will be stored in sealed plastic bags and transported to the laboratory in an insulated container. Before processing, all the samples are sieved (<2mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample.

- **Isolation of vesicular arbuscular mycorrhiza species:** From the above collected soil samples the spores of VAM fungi will be isolated by the wet sieving and decanting technique. (Gerdemann and Nicolson, 1963).

- **Identification of vesicular arbuscular mycorrhiza species:** VAM fungal spores will be examined and counted under stereomicroscope and identifications will be made by observing diagnostic characteristics such as spore wall, colour, size and type of hyphal attachment (according to Schenck and Perez, 1990).The synoptic keys of Trappe (1982) will be used to study the genera and species of Zygomycetous mycorrhizal fungi.

- **Data analysis of field collected sample:** This will be done for species richness of Arbuscular mycorrhizal fungi, frequency analysis and Relative abundance of VAM species from the collected soil samples. This will be done by using following formulae:

  Species Richness= number of Arbuscular mycorrhizal fungal species in 50gm air dried soil

  Frequency= (Number of zones in which Arbuscular mycorrhizal fungal species will be observed / Total number of zones) ×100%

  Relative abundance= Number of spores of an Arbuscular mycorrhizal fungal species at one site / Total number of spores at that site ×100%
  After doing data analysis the most predominant VAM species will be cultured by single spore culture technique (Nicolson, 1967).Here spore of fungi will be taken in water and will be picked up with fine capillary pipette under dissecting microscope. A single spore will be placed in the neck region of a funnel assembly filled with

sterilized sand and established seedling of *Sorghum bicolor*. The seedlings will be maintained in funnel for four weeks and then transferred to the mud pots containing sterilized 1:1 sand soil mixture. The pure isolates of VAM will now be multiplied in sterile conditions. The root system of uniformly infected sorghum seedling together with adhering soil will be used as starter culture.

- **Analysis of soil samples:** As it is known fact that VAM association with plant is related to the physiochemical and biological properties of the land therefore analysis of soil samples will be done on various parameters like pH (digital pH meter), estimation of Chloride ion, Heavy metals, Organic Carbon by Chromic Acid Method (Datta *et al.*, 1962), Nitrogen by Kjeldahl Method (Jackson, 1973), Phosphorous Estimation by Olsen's Method (Olsen *et al.*, 1954).
  The next step of experiment will be carried out in mud pot .Here the barren land soil samples (without VAM Fungi) will be transferred to the mud pots. Before transferring, the soil will be steam sterilized for 2 hours for two consecutive days. The sterile soil in pots will be inoculated with 5-10% of starter inoculums as a layer of two inches below the soil level and surface sterilized seeds of seasonal crops (one winter and one summer) will be sowed. The seedlings will be periodically watered and the relative efficiency of different VAM species will be studied on various growth parameters (viz. Morphological, Biochemical and Histochemical) along with control conditions.

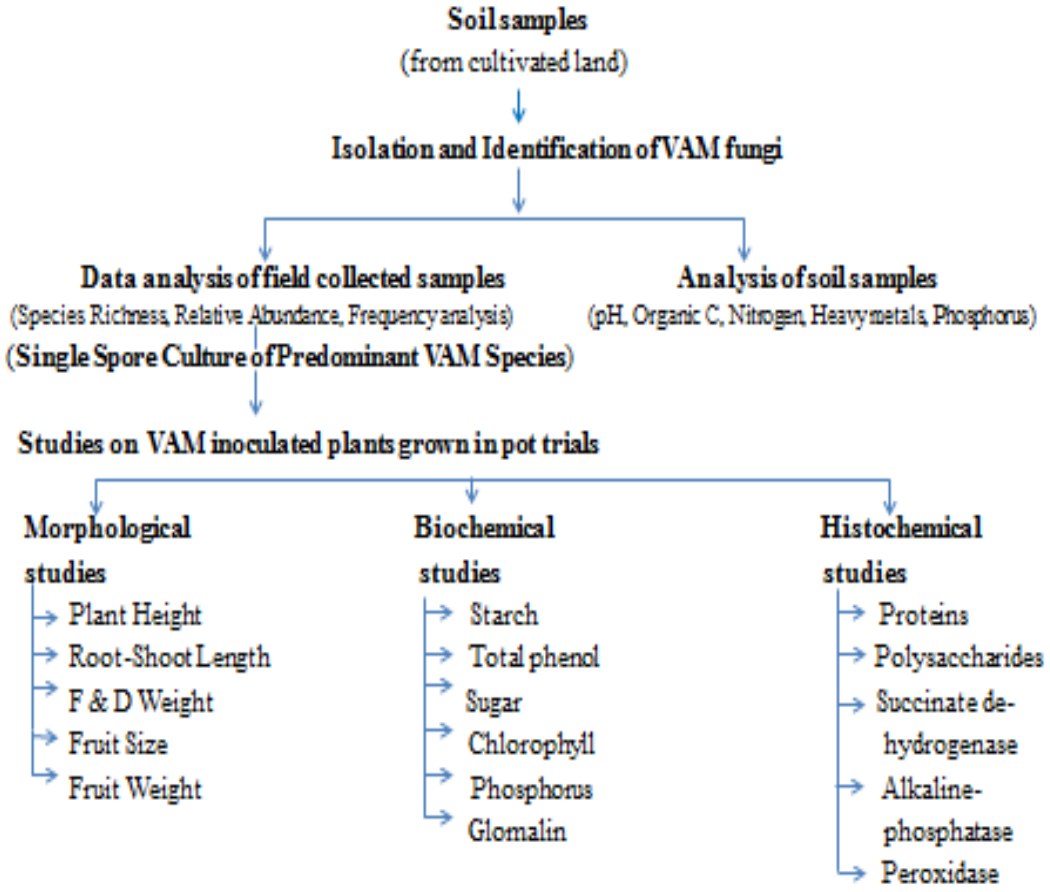
- **Morphological studies:** This will be carried out on plant inoculated with different vesicular arbuscular mycorrhiza fungi in pot trials. The plants will be harvested after 90 days of growth and observations will be made on different parameters like plant height, shoot length, root length, Fresh weight and dry weight of leaf, root and stem, fruit size and percentage mycorrhizal infection(Phillips and Hayman, 1970).

- **Biochemical Studies:** The quantitative and qualitative estimation of various biochemicals will be done on same plants. The various test are as follows:-
  -Estimation of Starch (Chandra mohan et *al.*, 1973)
  -Estimation of total Phenol (Bray and Thorpe, 1954)
  -Estimation of reducing sugar (Nelson, 1994)
  -Estimation of non-reducing sugar (Inman, 1962)
  -Extraction and estimation of Chlorophyll (Arnon, 1949)
  -Estimation of phosphorous (Jackson, 1964)
  -Quantitative estimation of Glomalin (Wright and Upadhayaya, 1996).

- **Histochemical studies:** This will be done on same Vesicular arbuscular mycorrhiza inoculated plants. Histochemistry is the localization of the chemical substances inside the tissue. For this root inoculated with VAM are selected and its transverse sections are prepared. The microscopic sections show the presence of

fungal components inside the tissue. With the help of different staining reactions, localization is made to establish the chemicals like polysaccharides, enzymes (Succinate dehydrogenase, alkaline phosphatase, and peroxidase) and proteins.

OUTLAY



YEAR WISE PLAN OF WORK

1-24 months	Review of literature throughout the study
1-3 months	Sample Collection
3-6 months	Identification of VAM Fungi and analysis of soil samples
6-12 months	Experimental work (**I)** pot trials and study of morphological characters
12-20 months	Experimental work (**II)** Biochemical and histochemical studies in same plants
20-24 months	Thesis preparation

Total Time (expected) = 24 Months

WORK PLAN (BAR CHART)

Activity / Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Review of literature																								
Sample collection																								
Identification of VAM fungi and soil sample analysis																								
Study of morphological characters (under pot trials)																								
Study of Biochemical, histochemical parameters in same plants																								
Thesis preparation																								

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Web Links:-

www.mycorrhizas.info

www.agricoop.nic.in

en.wikipedia.org/wiki/Arbuscular mycorrhiza